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NUTRITION REVIEWS

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NUTRITION REVIEWS

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NUTRITION EDUCATION

Nutrition education is the "retail end" of nutrition research. Through educational methods and technics, the information gleaned from laboratory research is filtered out to acquaint the public with information for more healthful living. "Nutrition education" is different from "food education." The latter is familiar. Its mediums are television, the radio, billboards, newspapers, magazines, and hearsay. The public accepts a surprising amount of this type of education. Its weakness is that it is often short-lived.

On the other hand, nutrition education takes in a broader scope. It aims to improve the health and nutritional status of the American people of all ages, occupations, and walks of life through interpretation of research. To obtain results, it would be necessary for either the persons involved to be eager and willing for a change of dietary habits, or for their daily food habits to be sufficiently pliable that they could be molded into more healthful ones. The first group is few in number. The latter, especially if one considers the several millions of schoolchildren in America today, is an enormous, potential, untapped faction.

If nutrition education could reach all children from 6 to 16 years of age, and if it could be included as part of a regular school curriculum throughout the country, it would bring two results: (1) Every boy and girl of school age would have an opportunity to learn about basic nutrients and basic foods and how they contribute to his daily and total health. Though the pattern of a child's food habits has been established by the time he enters school, it has not been so firmly entrenched that he rebels at changing it. The desire to please and the pressure of the group to which he belongs contribute a great deal to the changing process. (2) Many administrators and teachers would drop their primary criticism, that nutrition education is an "extra subject," since it would be included as regular classroom work. It is recognized that the school day is packed with curricular and extracurricular activities. To insist that nutrition take the place of other subject matter, would bring protest. Moreover, there is no need for such a recommendation. Nutrition education can be, and is, a part of health education, science, social studies, reading, arithmetic, and writing—all subject areas which, it is hoped, every child is receiving.

Through these divisions of the curriculum, it is simple and logical to include basic information and experiences regarding everyday nutrition and diet. The information and classroom activities will vary with the grade levels. However, regardless of the grade, age, or learning ability of the child, nutrition education should be based on the needs of the group. In general, in the early elementary grades (kindergarten through third grade), it is important that the information and experiences acquaint children with the basic food groups needed every day for a healthful diet, with the varieties of foods making up the American diet, and with the importance of food conservation and of cleanliness and sanitation in relation to food and eating. This "exposure" to a good foundation for habits and attitudes comes at a time when food habits are in a flexible and formative stage. At this age, a child is greatly interested in the growth and development of his own body, and is not so intrigued with the "muscle and prowess" stage that comes later when he becomes more group conscious. Later, he becomes more determined to know "why" he should eat certain foods and "what" they do for his body. Thus, at the later elementary grades

(fourth through sixth grades), he is ready and able to grasp more of the factual information in relation to nutrients and food.

To coordinate nutrition education with the standard curriculum, it is necessary for a school faculty to understand the basic groundwork in health and nutrition that should be stressed at different age levels. In an effort to assist this important phase of a school nutrition program, there has been developed in the Department of Nutrition at the Harvard School of Public Health an outline entitled "Goals for Nutrition Education for Elementary and Secondary Schools."* The purpose of this outline is to serve as a foundation for planning a coordinated program, not only within the elementary grades or the secondary grades, but, more important, as a total program from kindergarten through twelfth grade. Only thus will the ultimate goals of better nutrition and better dietary habits for young Americans be achieved. A second pamphlet, "Activities in Nutrition Education for Kindergarten through Sixth Grade,"** has been prepared. This booklet relates some of the school activities and illustrates some of the methods and technics by which teachers have incorporated nutrition education into daily classroom activities.

A school cafeteria is the "proving-ground" for testing acquired attitudes and knowledges. A functioning relation between the cafeteria and the classroom is essential. In fact, many school programs in nutrition education are rightfully focused around the school cafeteria. As one example of coordination, when new foods are to be introduced in the lunchroom, the classroom teacher can discuss them with her group, letting them sample a small bit for texture and flavor, and talk about the preparation and methods of serving. Children are then prepared for the appearance of the new food, and are not so inclined to leave it on their plates because of unfamiliarity.

Consumer education in relation to food and diet should play a major role in all phases of nutrition education. Many small children are sent to the stores daily for a loaf of bread, a can of corn or soup, or a bunch of carrots, hence they can learn and profit from consumer education. Availability of foods, condition of fresh fruits and vegetables, comparison of can sizes, cost of items—all enter into the economic backlog which every home needs to consider. Why wait until a child is an adult to learn about these vital issues when they can be made a part of his arithmetic, social studies, and science at all grade levels from third or fourth grade on through the twelfth grade? While boys and girls obtaining courses in home and family living at the secondary school level will receive some of the information about consumer education, this group comprises a small percentage of the total school enrollment. If such experiences are not planned for the remaining numbers, their information and experiences are entirely dependent upon the home. Few parents take time for such training.

Often the example of a "good breakfast" or luncheon within the classroom can serve to stimulate better attitudes. These meals should be simple, and can be planned by the boys and girls themselves. Far too many children, as well as adults, omit a meal if hurried or busy. Breakfast is particularly important, since many of the surveys of dietary intake of schoolchildren show that this meal is often omitted or neglected. It is startling to contemplate the vast number of high school students alone who start the day on a cup of coffee and a roll or doughnut.

If a school program in nutrition education is to function and be successful, it is necessary that the teachers themselves have information. To teach children the rudiments of health and diet means that the

*Available from The Nutrition Foundation, Inc., Chrysler Building, New York 17, New York at 15¢ per copy.

**Available from The Nutrition Foundation, Inc., Chrysler Building, New York 17, New York at $1.00 per copy.

teachers first must have the essential knowledge. Very few teacher-training institutions require course work in health and nutrition. How then can teachers be expected to impart accurate and needed information? Teacher's colleges and schools of education must keep up with the times. The aim is not to make a nutritionist of every school teacher, but to teach the importance of good diet and nutrition, to show how such information can be introduced into the standard curriculum, and to supply the teacher with accurate teaching materials and with suggestions for methods and technics. This can be accomplished through preservice courses for teachers-to-be, and through in-service courses for teachers in the field. Superior training would give basic information regarding health, nutrition, and diet as an accompaniment to the course work usually required in child development and growth. This would be followed by courses in materials, methods, and technics for including health and nutrition education as a part of the daily curriculum. Teachers would then go into the classroom prepared to include nutrition education as an important phase of the health program.

Not only is it necessary for teachers to have essential knowledge regarding diet and nutrition, but it is also necessary for them to practice what they advocate. A child, particularly at the elementary grade levels, learns a great deal through observation. This improvement in teachers, rather than in teaching, may be more difficult to bring about. Adroit community and educational pressure can assist.

If these two groups—schoolchildren and school teachers—could be given planned nutrition education as a part of their health programs, within a comparatively short time there would be a nutrition-conscious people. Automatically, it would include all walks of life, all occupations, all ages. However, since nutrition knowledge is based on scientific research which is a constantly changing picture, the subject matter must permit changing more readily than do most school subjects. This problem, common to most areas of health education, can be made an item of added interest.

Nutrition education, when given with the regularity of the "three R's," would be permanent. Food habits would come to include a basis of scientific knowledge, as well as family characteristics, economic standing, racial background, community customs, and age. Then it might be possible to justify the claim that "Americans are the best fed people in the world."

ELIZABETH A. LOCKWOOD
Department of Nutrition
Harvard School of Public Health
Boston

XANTHOPTERIN IN ANIMAL NUTRITION

Since its initial discovery as a pigment in yellow butterfly wings, and subsequent observations of a much more widespread occurrence in nature, the possibility that xanthopterin plays some important physiologic role has intrigued many investigators. Especially has this been true since the discovery that the nuclear structure of this compound and that of folic acid (pteroylglutamic acid) were identical. A number of physiologic roles for this compound has been reported.

As early as 1936, R. Tschesche and H. J. Wolf (*Z. f. physiol. Chem.* **244,** *1* (*1936*); **248,** *34* (*1937*)) reported that xanthopterin would cure an anemia produced in rats by a diet of goat's milk, and in 1941 E. R. Norris and R. W. Simmons (*J. Biol. Chem.* **140,** *679* (*1941*); **158,** *449* (*1945*)) reported that this substance when injected into anemic

fish produced a rapid rise in red blood cells, which returned to normal levels. J. R. Totter and P. L. Day (*Ibid.* **147,** *257 (1943)*) reported that xanthopterin alleviates the leukopenia observed in rats fed a purified diet containing succinylsulfathiazole. The latter observations, however, could not be confirmed by several groups of workers (L. D. Wright and A. D. Welch, *Science* **98,** *179 (1943)*; F. S. Daft and W. H. Sebrell, *Pub. Health Reports* **58,** *1542 (1943)*) who found, however, that leukopenia induced in this manner was alleviated by administration of folic acid. Although the antianemic properties of the latter substance have been intensively investigated in recent years, those of xanthopterin have received comparatively little attention, and, with exceptions noted above, have been neither confirmed nor denied.

The effects of xanthopterin have now been further investigated with results summarized in a series of reports by E. R. Norris and J. J. Majnarich. In the first of these (*Am. J. Physiol.* **152,** *175 (1948)*), bone marrow was aspirated from the sternum and ribs of rabbits, diluted in Tyrode's solution (without glucose), and centrifuged to remove coarse material, debris, and emulsified lipids. The resulting bone marrow suspension was distributed in equal volumes to a medium containing casein hydrolysate and tryptophan, these tubes were then supplemented with xanthopterin, folic acid, or normal blood serum. Unsupplemented cultures were retained as controls. Red blood cell, white blood cell, and reticulocyte counts were made at zero, three, and six hours after supplementation, and the increases in the various cell types recorded at the two time intervals. Striking increases in cell counts were observed with all three cell varieties in those cultures supplemented with either xanthopterin or with normal blood serum. Folic acid produced essentially no increases over those observed in the unsupplemented control tubes. As illustrative of the results obtained, the following figures indicate the initial and final red blood cell counts in the various groups: (a) unsupplemented controls, 21×10^4 and 23×10^4; folic acid (5 micrograms per milliliter) 21×10^4 and 24×10^4; xanthopterin (5 micrograms per milliliter) 21×10^4 and 32×10^4; blood serum (0.1 ml. per milliliter) 21×10^4 and 37×10^4 cells per cubic millimeter. Variations in the concentration of xanthopterin showed a definite optimum concentration at approximately 5 micrograms per milliliter; higher amounts of this compound suppressed proliferation.

In their second paper (*Am. J. Physiol.* **152,** *179 (1948)*) weanling rats were made anemic by feeding a synthetic-type diet containing 1 per cent of sulfathiazole. At twenty-two days, the average red blood cell count was 3.4×10^6 per cubic millimeter, and the average hemoglobin 6.8 g. per 100 ml. At this time each rat received 100 micrograms of xanthopterin per day for five days by injection. Pronounced increases in red blood cell count (to about 9×10^6 per cubic millimeter), hemoglobin (to 15 g. per 100 ml.), and hematocrit (from 20 to about 43 ml. per 100 ml.) occurred during the twenty to thirty days following initiation of these injections.

In subsequent experiments, the response to injections of synthetic xanthopterin (100 micrograms) and synthetic pteroylglutamic acid (folic acid) was compared. As before, an immediate response to xanthopterin was noted. A response to folic acid quantitatively similar in magnitude was observed, but only after a delay of three to five days. Thus, whereas an immediate response to xanthopterin was noted, that to folic acid occurred only after a lag period. From the observation that folic acid was ineffective in stimulating proliferation of cells in bone marrow, whereas xanthopterin was immediately active, the authors attribute the lag observed with folic acid to the necessity for degradation of this substance to xanthopterin before it can be utilized by the bone marrow in erythropoiesis.

Impressed by the definite optimum in

xanthopterin concentrations which was required to permit rapid cell proliferation in bone marrow, the same authors (*Am. J. Physiol.* **153,** *133 (1948)*) determined the comparative effects of various high levels of xanthopterin in curing anemia in rats. Anemia was produced by feeding the diet described above, and the xanthopterin was administered as a single dose by injection. Under these conditions, a dose of 1 mg. of xanthopterin per kilogram of body weight produced a spectacular rise in red cell count, reticulocyte count, white cell count, hematocrit, and hemoglobin concentration, all of which increased regularly for ten to fifteen days following injection, then decreased slowly. An injection of 5 mg. of xanthopterin per kilogram of body weight was somewhat less effective. Levels of 10 and 20 mg. of xanthopterin were, however, without favorable effect upon the blood picture; in fact, they intensified the anemia. Thus, in the rat, as in isolated bone marrow, a definite optimum in xanthopterin concentration was observed, and it is possible that the failure of some groups to observe a favorable effect of xanthopterin under similar conditions resulted from administration of a level outside this optimum range.

Again, under these conditions, a comparison revealed folic acid to be somewhat less active than xanthopterin in curing anemia at levels equimolar with xanthopterin, when the latter was administered in optimum amounts. In normal rats, the optimum effective levels of xanthopterin produced a polycythemia and leucocytosis. Dosages higher than 5 mg. per kilogram of body weight, however, produced anemia and leukopenia.

These highly interesting and suggestive results indicate the need for a careful reexamination of the relation between folic acid and xanthopterin in metabolism. Both compounds occur naturally, and are of similar structure. Do they function independently, or is xanthopterin in fact an "active" form of folic acid?

FAILURE OF CORRELATION OF GROWTH REQUIREMENTS FOR NIACIN, NICOTINAMIDE, AND COENZYME I

The niacin requirement of a microorganism can often be satisfied by nicotinamide or coenzyme I (diphosphopyridine nucleotide, DPN). The general assumption is that microorganisms using these substances convert niacin to nicotinamide and use the latter to form coenzyme I. Contradictions to this general principle have been studied by S. A. Koser and G. J. Kasai (*J. Infect. Dis.* **83,** *271 (1948)*).

B. C. Johnson (*J. Biol. Chem.* **159,** *227 (1945)*) pointed out that Leuconostoc mesenteroides 9135 required niacin for growth but that such growth was not supported by nicotinamide at ordinary levels. Previously the reverse situation had been found for some bacteria of the Pasteurella group, namely a requirement for nicotinamide, while niacin was not effective in supporting growth (S. A. Koser, S. Berkman, and A. Dorfman, *Proc. Soc. Exp. Biol. Med.* **47,** *504 (1941)*).

Employing customary technics, Koser and Kasai (*loc. cit.*) observed that two Leuconostoc cultures required niacin for growth but failed to grow in the presence of coenzyme I or nicotinamide when these compounds were supplied in ordinary vitamin amounts. A third Leuconostoc strain grew well in the presence of niacin and coenzyme I but did not grow when nicotinamide was supplied. A sample of coenzyme II in a limited number of tests gave results similar to those obtained with coenzyme I. Unusually large amounts of coenzyme I or nicotinamide supported slow growth at 24° and 30°C. but not at 37° C. Niacin, on the other hand, supported prompt, vigorous growth at 37°C. as well as at the lower temperatures. Even in the

presence of niacin, nicotinamide did not noticeably accelerate or retard growth. Finally, methyl nicotinate and nicotinuric acid, compounds which possess a high degree of growth-promoting activity for other niacin-requiring microorganisms, exhibited little growth-promoting activity for the three Leuconostoc cultures.

These unexpected observations raise the question of how niacin is utilized by the cells. Difficulties of penetration of the cell membrane by coenzyme or nicotinamide do not seem to be significant since with all known niacin-requiring microorganisms, preformed coenzyme and nicotinamide exert prompt metabolic effects in either growth tests or with cell suspensions. It would seem unlikely that there was present in the basal medium some substance acting as a metabolic antagonist which was capable of blocking nicotinamide and coenzyme but not niacin. The same environment used in the studies of Koser and Kasai did support the growth of several other closely related Leuconostoc strains when nicotinamide was supplied.

The three cultures in question are apparently unable to convert nicotinamide to niacin or do so only with difficulty since they fail to grow when the amide replaces the acid in ordinary vitamin amounts and grow slowly only when considerably larger amounts of nicotinamide and coenzyme are present. Whether this latter situation represents a gradual conversion by the cells of the amide and coenzyme to niacin in amounts sufficient to support slow growth is not known. The inability to make use of methyl nicotinate which is readily utilized by other niacin-requiring bacteria also may be interpreted as pointing to a more limited capacity on the part of these organisms to change closely related structures to niacin or to an active form.

It seemed possible that niacin is used by the cells for some purpose other than formation of coenzymes I and II (DPN and TPN). However, Leuconostoc cultures grown in the presence of niacin do produce a substance which supports growth of Hemophilus parainfluenzae, the usual method for demonstrating the presence of coenzyme. While it is possible that a coenzyme similar in structure to DPN or TPN is synthesized from niacin rather than from the amide, it is then necessary to assume that Hemophilus is able to change this hypothetical coenzyme to DPN by changing the niacin portion of the molecule to the amide.

Another explanation which has been proposed is that a niacin riboside is formed which is subsequently converted to nicotinamide riboside rather than formation of riboside from the combination of nicotinamide and ribose. Such a mechanism might account for the behavior of culture 9135 which responded to coenzyme I but not to nicotinamide.

Still another explanation is that niacin may be used in more than one metabolic pathway. One such use would be for the usual coenzyme formation. A second use would have to be assumed for construction of some unidentified niacin complex, compound X, which may or may not be structurally related to DPN or TPN. This compound or its niacin precursor is required for growth. The organisms cannot convert nicotinamide to niacin or to the unidentified niacin complex, so in this case the acid is necessary and nicotinamide is of no help for this particular process. If the unknown substance were known and supplied preformed to the cells, then one might find that these Leuconostoc cultures would respond to added nicotinamide or coenzyme I as well as to niacin. Failure of growth in the presence of nicotinamide or coenzyme is, under this assumption, due to deficiency of another substance, compound X, or its precursor, niacin, and also to inability of the cells to convert nicotinamide to niacin.

As the authors point out, whatever may be the explanation for the discrimination exhibited by the three Leuconostoc cultures, it seems probable that present ideas of the metabolic use of niacin will have to be modified or extended.

CONVERSION OF VITAMIN A TO CAROTENE

Since the recognition that beta-carotene is a precursor of vitamin A, problems of where and how it is converted into vitamin A in the animal body have been discussed and explored experimentally. Some studies indicated that the intestinal wall and not the liver is the most likely site in which this conversion takes place (*Nutrition Reviews* **6,** *92 (1948)*). This postulate was based on the ability to detect vitamin A or a vitamin A-like substance by the Carr-Price reagent in the intestinal wall prior to detecting it in the liver of rats which had been given beta-carotene by stomach tube after a period of vitamin A depletion. Data were also presented to indicate that vitamin A or a vitamin A-like compound, as detected by the Carr-Price reagent, was formed from beta-carotene *in vitro* when portions of the small intestine of vitamin A deficient rats were incubated in Ringer-Locke solution under anaerobic conditions.

F. H. Mattson (*J. Biol. Chem.* **176,** *1467 (1948)*) disclosed that the Carr-Price reaction was not specific for vitamin A. Evidence was presented which indicated more conclusively that the compound formed under these experimental conditions was vitamin A. Four hours after 1200 micrograms of beta-carotene in cottonseed oil were administered to each of 10 vitamin A deficient rats, the rats were killed and their intestinal tracts were removed and then washed with isotonic saline solution. The intestines were treated in such a way that any vitamin A or vitamin A-like compounds would be extracted. These fluorescent compounds were purified sufficiently that a determination of their absorption curve could be made. Comparison of this absorption curve with the absorption curve of vitamin A alcohol indicated that the two were similar. In addition, mixture of this fluorescing compound with vitamin A alcohol resulted in a single fluorescing band when a chromatogram of the mixture was made and developed. These data identify qualitatively the presence of vitamin A *per se* in the intestinal wall of the rat under these experimental circumstances and remove doubt as to whether compounds other than vitamin A might have been determined by the nonspecific Carr-Price reaction.

In earlier studies, E. L. Sexton, J. W. Mehl, and H. J. Deuel (*J. Nutrition* **31,** *299 (1946)*) demonstrated that the intestinal wall of the rat appeared to act as a barrier beyond which beta-carotene could not pass and that suspensions of beta-carotene introduced into the bodies of vitamin A deficient rats by intravenous, intraperitoneal, or intrasplenic injection accumulated in the liver without increase in the vitamin A content of the liver or of the plasma. High amounts of beta-carotene were still detectable in the liver of vitamin A deficient rats for as long as forty-six days after injection. Despite the relatively large amounts of beta-carotene which remained in the liver after parenteral injection, this precursor of vitamin A appeared to be useless to the vitamin A deficient rat. A paradoxical situation had been produced when rats were dying of vitamin A deficiency even though their livers contained sufficient beta-carotene to maintain life and limited growth for periods of a year or more if that same amount of beta-carotene had been administered at a level of 0.5 microgram daily via the digestive tract. For example, the livers from 2 rats which had died on the forty-fourth day after the intrasplenic injection of 592 micrograms of beta-carotene contained 185 and 214 micrograms of carotene, respectively. There was no question about the chemical identity of the carotene in the liver, for all tests indicated that it was identical in structure with the injected material. Furthermore, since rats with high deposits of carotene in the liver were able to utilize beta-carotene administered orally, no fundamental disorder in carotene metabolism could have existed in the rats because of the abnormally high carotene content of their livers.

When beta-carotene was given orally in cottonseed oil or in its naturally occurring relation in alfalfa, vitamin A levels of the liver and plasma of vitamin A deficient rats increased within a few hours. Unlike the livers of the rats in which beta-carotene had been injected, not a trace of carotene could be detected in the liver or plasma of rats which had been given beta-carotene through the oral route.

The site of conversion of carotene to vitamin A and the ability of the intestinal wall to transfer carotenoids from the gut across its cells to the lymphatics may vary from one species to another. Although carotenoids are not ordinarily present in the plasma of the rat, substantial amounts are routinely observed in bovine plasma and large amounts are secreted into the milk. P. D. Boyer *et al.* (*J. Dairy Sci.* **25,** *433* (*1942*)) observed that the carotene content of the plasma of Holstein and Guernsey calves varied roughly in proportion to the amount of carotene ingested and reached levels of at least 58 micrograms per 100 ml. of plasma in the Holstein and at least 137 micrograms per 100 ml. of plasma in the Guernsey. These increases in carotene content were accompanied by increases in the vitamin A content of the plasma up to about 10 micrograms per 100 ml. of plasma. In this investigation, parenteral administration was not attempted to test whether the bovine species was able to convert beta-carotene, which was already in the circulatory system, into vitamin A.

ON THE IDENTITY OF VITAMIN B_{12} AND THE ANIMAL PROTEIN FACTOR

The animal protein factor has become recognized as a factor associated with various animal protein sources, but lacking in vegetable protein sources. The factor was recognized by J. C. Hammond (*Nutrition Reviews* **4,** *116* (*1946*)) in studies of chick growth. It appeared that dried cow manure, dried rumen contents, or sardine meal increased chick growth when the basic feed contained only vegetable sources of protein. H. R. Bird and co-workers (*Ibid.* **4,** *330* (*1946*); **6,** *71* (*1948*)) distinguished the factor in cow manure from known nutrients and established its independent nature. The factor was concentrated from cow manure some one hundred fold, and liver was recognized as a good source. The chick growth factor in cow manure was apparently the same as the factor found to improve egg hatchability when hens were maintained on vegetable protein rations. It may also be the same as the "zoopherin" described by L. M. Zucker and T. F. Zucker (*Ibid.* **6,** *219* (*1948*)) as an essential for rats, its deficiency being noted again when the dietary protein was entirely of plant origin. The animal protein factor concerned in this review appears different, however, from the lipid soluble "animal protein factor" referred to by G. F. Heuser, L. C. Norris, H. L. Lucas, and G. F. Combs (*Poultry Sci.* **25,** *403* (*1946*)) and by E. L. Johnson, C. W. Carrick, R. E. Roberts, and S. M. Hauge (*Ibid.* **21,** *566* (*1942*)). Microbiologic assay of the chick growth or animal protein factor has been reported by H. R. Skeggs, J. W. Huff, L. D. Wright, and D. K. Bosshardt (*J. Biol. Chem.* **176,** *1459* (*1948*)) using Lactobacillus leichmannii.

Vitamin B_{12} is recognized as a factor which is derived from liver and which is potent in the treatment of pernicious anemia (*Nutrition Reviews* **6,** *245, 291* (*1948*)). This factor has also been isolated from Streptomyces griseus by E. L. Rickes and co-workers (*Science* **108,** *634* (*1948*)).

Recent evidence indicates strongly that vitamin B_{12} and the animal protein factor are identical. In a comparative study of Lactobacillus lactis Dorner and L. leichmannii, W. Shive, J. M. Ravel, and W. M. Harding (*J. Biol. Chem.* **176,** *991* (*1948*)) found that thymidine replaced refined liver

extract in the growth of both organisms. Since the former organism was that used in concentration of vitamin B_{12}, and the latter responds to highly purified concentrates of the animal protein factor, the close relation between the latter and vitamin B_{12} becomes apparent. Thymidine replaced the growth requirements of the respective factors in these organisms, and the difference between these factors and folic acid was evident. More direct evidence for the identity of vitamin B_{12} and the animal protein factor was provided by W. H. Ott, E. L. Rickes, and T. R. Wood (*Ibid.* **174,** *1047 (1948)*) who demonstrated that vitamin B_{12} had growth activity for chicks maintained on a soybean protein diet such as that used by Bird and associates in their studies of animal protein factor. R. J. Lillie, C. A. Denton, and H. R. Bird (*Ibid.* **176,** *1477 (1948)*) have furthermore tested vitamin B_{12} in direct comparison with their purified cow manure growth factor and with liver extract. The chick growth response was similar with these preparations, and the conclusion seemed evident that the factors were identical, although since the chick growth factor has not yet been obtained in the pure state, it is still possible that different forms of the vitamin exist. Vitamin B_{12} exerted its growth effects orally or after intramuscular injection, hence its effect is direct and not mediated through intestinal bacteria. Skeggs, Huff, Wright, and Bosshardt (*loc. cit.*) have shown that growth of L. leichmannii is stimulated under the same culture conditions by a purified animal protein factor preparation and by liver extracts. Thymidine replaces the requirement of either preparation. Thus it seems clear that the animal protein factor and vitamin B_{12} are either identical or very closely similar.

Crystalline antipernicious anemia factor (vitamin B_{12}) and liver extract produce the same maximum growth response with L. leichmannii (C. E. Hoffmann, E. L. R. Stokstad, A. L. Franklin, and T. H. Jukes, *J. Biol. Chem.* **176,** *1465 (1948)*), although further clinical data seem necessary to determine whether all of the activity of liver extract in the treatment of pernicious anemia can be accounted for by vitamin B_{12}.

AMINO ACID EXCRETION IN HUMAN URINE AND FECES

The relation between the quality of protein ingested by mice and rats and the urinary excretion of free and combined amino acids has been investigated (*Nutrition Reviews* **7,** *44 (1949)*). For example, when the protein was deficient in methionine but otherwise complete, the amounts of ingested amino acids excreted by mice varied from 24.6 per cent of the glutamic acid to as much as 86.5 per cent for histidine. In contrast, when the source of protein in the ration was adequate in the form of blood fibrin at a level of 20 per cent, the amounts of ingested amino acids excreted in the urine were much lower and ranged from 0.8 per cent for arginine to 5.4 per cent for histidine.

Among the studies of amino acid excretion in human urine are those of A. L. Sheffner, J. B. Kirsner, and W. L. Palmer (*J. Biol. Chem.* **175,** *107 (1948)*). Two young men who were believed to be healthy so far as could be determined by a variety of diagnostic tests were selected as subjects. They were hospitalized in a metabolic ward to permit accurate control of food intake and collection of urine. The diets contained approximately 1 g. of protein per kilogram of body weight per day and sufficient calories to prevent the need to utilize protein unduly as a source of energy. The balance periods were of six days duration and were preceded by a transition period of maintenance for three days on the diet to permit standardization of the subjects to the regimen. Rep-

resentative aliquots of the twenty-four hour urine samples were autoclaved in dilute hydrochloric acid for ten hours. Amino acid assays were made by microbiologic procedures.

The quantity of amino acids excreted in the urine, averaged for the 2 subjects over the entire period, ranged from 0.43 per cent of the amount ingested for leucine to 12.65 per cent for histidine. For the eight amino acids determined, an over-all average of 2.46 per cent of the amounts ingested was excreted in the urine. The quantities of these amino acids excreted in the urine during any twenty-four hour period were in good agreement with the values reported by other investigators (*Nutrition Reviews* **6,** *6 (1948)*; H. W. Woodson, S. W. Hier, J. D. Solomon, and O. Bergeim, *J. Biol. Chem.* **172,** *613 (1948)*). In addition, the percentages of amino acids excreted in the urine of these human subjects were comparable to the percentages excreted by mice maintained on a diet with an adequate source of protein. The subjects in this experiment were in slightly positive nitrogen balance and maintained their weight throughout; thus, no inadequacies in the diet were demonstrable in this short period.

The determinations of the amino acid concentrations in plasma were made so that the renal clearance of the individual amino acids (that is, the milliliters of plasma freed or cleared of a particular amino acid in one minute by renal elimination) could be calculated. With the exception of methionine, hydrolysis of the plasma tungstate filtrate resulted in large increases in the microbiologically available amino acids. Relative increases in the various amino acid values did not coincide with the amino acid concentration of total plasma proteins, plasma albumin, or plasma fibrin. The increases were likely due to the hydrolysis of peptides, polypeptides, or amino acid esters as most of the higher molecular weight complexes had been precipitated in the preparation of the tungstate filtrate. The total concentration of any amino acid in the plasma tungstate filtrate was used in the calculation of the renal clearance for that particular amino acid. A large variation was observed in the relative renal clearance. The average renal clearance for the 2 subjects was less than 1 ml. of plasma cleared per minute for leucine, isoleucine, valine, and arginine, and between 1 and 2 ml. for threonine, lysine, and methionine. In contrast, the average renal clearance was 9.59 ml. of plasma per minute for histidine. This variation demonstrated the wide selectivity of the renal tubules in their normal reabsorptive function.

A. L. Sheffner, J. B. Kirsner, and W. L. Palmer (*J. Biol. Chem.* **176,** *89 (1948)*) also studied the rate of excretion of amino acids in the feces of the 2 subjects used in the previous investigation. The intake of protein was varied widely but the caloric intake and the weight of dried matter were kept as uniform as possible by adjustment of the carbohydrate content of the diet. The amount of individual amino acids ingested was varied by changing the quantity or quality of the proteins in the diet except in one instance when large amounts of methionine were added. Care was taken in the design of the experimental diets so that they would be adequate except for modifications in the protein content. Each diet was studied for two consecutive six day periods, preceded and followed by a three day interval which permitted adjustment to the new diet. The rates of excretion were measured for the same eight amino acids studied in urine previously. These amino acids were made available microbiologically by autoclaving a suitable aliquot of the feces in dilute hydrochloric acid for ten hours.

The amount of nitrogen excreted in the feces remained relatively constant throughout the investigation even though the amount of nitrogen ingested varied considerably. The amounts of amino acids excreted did not vary to any significant extent be-

tween the 2 subjects. Throughout the five tests with five diets having different amino acid distributions, the level of each of the eight amino acids in the feces remained remarkably constant. For example, the amount of histidine ingested varied least and ranged from 6.9 to 13.8 g. for the six day periods; the intake of lysine varied most and ranged from 13.2 to 31.7 g. per period. Despite these rather wide variations, the excretion of each amino acid did not increase or decrease with the intake. Thus, when 13.2 g. of lysine were ingested in each of two successive six day periods, 2.1 and 2.6 g. were excreted in the feces, representing the respective periods; when 31.7 g. of lysine were ingested in each period, 2.9 and 2.6 g., respectively, were excreted. To test whether addition of crystalline amino acids would alter this constant relation, 6.0 g. of DL-methionine were added during each of two successive periods to a diet already supplying 8.8 g. of methionine. No increased excretion of methionine occurred in the feces. On the other extreme of methionine intake, one of the subjects was later given a diet in which the methionine content had been reduced by hydrogen peroxide treatment of the peptone solution that was the major source of nitrogen. The total amount of methionine ingested in the two successive six day periods was 2.3 g. in the first and 0.9 g. in the second. The amount of methionine excreted during these periods was not reduced below the lowest values determined in the major investigation.

Tests indicated that essentially none of the fecal amino acids was present on excretion as free amino acids or in polypeptide form. The constancy of the excretion of amino acids in the feces eliminated almost all possibility that these amino acids were unabsorbed residues of dietary proteins. The results strongly suggested that the amino acids were excreted in the form of proteins, the composition of which had not been influenced by the composition or amounts of the proteins in the diet. Some of these proteins undoubtedly were bacterial in origin; others may have been products of the digestive tract, secreted into the lumen from which they might have been excreted as such or utilized for the nutriment of the intestinal flora and combined into bacterial cells.

These studies on the excretion of amino acids in human urine and feces supplement present knowledge about the metabolism of amino acids and proteins. Comparable to data obtained in rodent investigations, the urinary excretion of amino acids in man was low when the protein content of the diet was adequate. The present studies did not test whether the excretion of amino acids in human urine would increase dramatically when incomplete proteins were fed as has been demonstrated for rodents. Unlike the amino acid content of urine, the distribution of the same amino acids in feces did not vary in amount with large increases or drastic decreases in the quantities of amino acids ingested.

NUTRITION IN PREGNANCY IN BRITAIN

The nutritional requirements and optimal food allowances in pregnancy, far from being settled, are still discussed by experts in the field. For example, the National Research Council recommends an allowance of 2400 calories daily during the latter half of pregnancy (*Nutrition Reviews* **6,** *319* (*1948*)), while in contrast, W. J. Dieckmann (*Ibid.* **6,** *129* (*1948*)) describes the diet recommended for a normal pregnancy at the Chicago Lying-In Hospital as containing 1808 calories daily. Authorities agree, however, that approximately 85 g. of protein should be allowed daily and, in general, the recommendations for minerals and vitamins have become standard. The recommendations of the Food and Nutrition Board of the National Research Council are usually

thought to be ample. B. S. Burke and H. C. Stuart (*Ibid.* **6,** *299 (1948)*) point out that although these allowances may be adequate during normal pregnancy, they presuppose that pregnancy begins with the woman in good nutritional status. If this is not the case, increased allowances may be required, a frequent occurrence in the patients studied by Burke and Stuart.

When adequate food is difficult to obtain, as for example in Great Britain during wartime rationing, one might expect stress groups, such as pregnant women, to suffer noticeably and early. To offset this, the British government made available carefully planned food supplements for pregnant women. These events produced marked alterations from prerationing days in the kind and quantity of food consumed. Two studies were carried out during the period of wartime rationing to analyze the quantity and quality of foods consumed during pregnancy. One study by W. Hobson (*J. Hygiene* **46,** *198 (1948)*) was carried out in Bristol and the other by E. Obermer (*Ibid.* **46,** *325 (1948)*) was made in London. The studies were compared with previous nutrition surveys of pregnant women, particularly with that of R. A. McCance, E. M. Widdowson, and C. M. Verdon-Roe (*Ibid.* **38,** *596 (1938)*), made in Great Britain just before the war.

The present study was conducted in London from 1943 to 1947 and was concerned with 55 average, healthy, working-class women aged 21 to 34 years who were in varying stages of pregnancy. The majority was said to live in slum areas. Each woman was given the appropriate apparatus and instructions so that she could weigh, measure, and record all food eaten for specified periods of time. In addition, frequent home visits were made by the observers.

As to caloric intake, there were two hundred and thirty-eight investigations in the 55 women. The mean caloric intake was 2482 with a wide range (1395 to 3938). The caloric intake rose strikingly during the second half of pregnancy: 26.3 per cent were consuming between 1500 and 2000 calories in the first half while during the second half only 10.3 per cent fell into this range. In the range 2500 to 3000 calories, 25.3 per cent were found in the first half and 41.8 in the second half of pregnancy. The same increase during the second half of pregnancy was noted for protein intake. For example, during the first half of pregnancy 16.3 per cent of the women recorded a diet containing from 50 to 70 g. of protein daily while during the second half the percentage on this low protein intake fell to 8.9. The percentage of women consuming considerably more protein, that is from 85 to 100 g. daily, rose from 33.8 per cent during the first half to 40 per cent during the second half of pregnancy. When one considers the National Research Council allowance of 85 g. of protein daily it can be seen that the majority of individuals consumed considerably less than the recommended allowance. In terms of grams of protein per kilogram of body weight, 57 per cent were above 1.5 g. daily. Strangely enough, although a pint of milk was allowed daily, only 60 per cent of the individuals made use of this supplement during the first half of pregnancy and only 48 per cent during the second half. The fat content of the diet was unexplicably high, particularly considering the scarcity of fat during wartime rationing in Britain. The mean fat intake was 107.4 g. daily. The carbohydrate content of the diet was unexpectedly low, 290.9 g. being the mean daily intake. The authors concluded that the food intakes were considerably below the recognized standards. They did compare favorably, however, with the prewar surveys of pregnant women, particularly with that of McCance, Widdowson, and Verdon-Roe (*loc. cit.*).

The study by Hobson in Bristol was of 111 normal pregnant women and was carried out during 1947. Four of the women were under three months pregnant, 96 between three and six months, and 11 between six months and

term. Food intake was measured and recorded much as done in the survey reported by Obermer. With regard to the articles of food, the consumption in 1947 was considerably greater than that observed in the 1938 survey although this was not true for butter or margarine or total meat consumption which fell off slightly during wartime rationing. The bread consumption increased from a mean of 48 ounces per week to 56 and milk from 63 to 123 ounces.

The intake of individual nutrients was compared with the National Research Council allowances for pregnant women and with the findings of McCance, Widdowson, and Verdon-Roe in 1938, where comparable figures were available. The mean caloric intake in 1947 and in 1938 was only slightly below that of the National Research Council allowance for pregnant women. However, in the later study 43 per cent of the women did not reach the American standard. The total protein intake in grams per kilogram of body weight daily increased from 1.2 to 1.5 from 1938 to 1947, the latter figure being identical with the National Research Council standard. Fifty-three per cent of this group reached the standard. The mean calcium intake increased strikingly from 1938 to 1947, rising from 0.7 to 1.2 g. per day. Still only 18 per cent of the 1947 group reached the American standard. Iron intake almost tripled from the earlier study and thiamine increased from 1.0 to 1.25 mg. per day. The other individual nutrients were not analyzed in the 1938 study but comparisons could be made with the National Research Council standards. The vitamin A intake was high (7600 I.U. as compared with 3500 I.U. for the National Research Council, due in a large measure to unspecified supplements). Intake of vitamin D approximated that suggested by the National Research Council, while riboflavin, niacin, and ascorbic acid were somewhat below this standard. The author discusses in considerable detail the adequacy of these diets and agrees with others who have pointed out that the National Research Council's recommended allowances are far too high a standard for Great Britain. Not one of the subjects studied was able to reach this standard in all regards. The authors comment, in fact, that the level recommended by the National Research Council is almost impossible to reach on present day incomes and availability of foods in Britain.

The possible sources of error in the study, such as the size of the sample, the influence of the study upon the individual's dietary habits, the length of the survey, and the period of pregnancy receive excellent discussion by the author. Analysis of these possible errors led to the conclusion that the study was probably as valid as could be obtained by any method. The method of assessing the food eaten was that described in the "Ministry of Foods Manual of Nutrition," *His Majesty's Stationery Office, London (1947)*.

In addition to the food analysis Hobson made a clinical survey of the patients. Only 8 per cent of the individuals had a hemoglobin level below 80 per cent of normal and only one woman had a plasma protein concentration below 5.5 g. per 100 ml. None of the usual signs suggesting nutritional deficiency was observed. Toxemia of pregnancy, however, occurred in 15 per cent of the cases. Although many of the subjects who developed toxemia consumed apparently adequate quantities of food, the majority had low intakes of bread and meat and thus of the vitamin B-complex, particularly niacin; in fact niacin was the only substance consistently low in the diets of the women with toxemia. The reduction in niacin intake of the individuals who had toxemia compared with those who did not have toxemia appeared to be statistically significant. The author quotes R. A. Ross (*Am. J. Obstet. Gyn.* **54,** *723 (1947)*) who in referring to the southern part of the United States said, "We have rarely found toxemia in the intelligent and adequately nourished

groups but it is the prime factor in the improperly nourished. In our areas such a patient would develop pellagra if exposed to the sun and we feel may develop symptoms of pregnancy toxemia if she becomes pregnant. The patients we see in eclampsic convulsions have come from the same group who subsisted on a diet similar to pellagra." The relation between inadequate food intake and the incidence of toxemia in pregnancy was carefully studied by B. S. Burke, V. A. Beal, S. B. Kirkwood, and H. C. Stuart (*Nutrition Reviews* **1,** *386 (1943)*) who found that 50 per cent of mothers whose diets were "poor" or "very poor" had toxemia.

Finally, the author pointed out that the most important cause for defective nutrition in these patients in whom it existed was ignorance, although lack of money and of food played a part. He concluded that an intensive nutrition education program was undoubtedly badly needed.

NUTRITIVE VALUE OF CANNED FOODS (PART I)

In 1942 a large-scale survey of the nutritive value of canned foods was begun under the joint sponsorship of the National Canners Association and the Can Manufacturers Institute. At the end of the first year progress reports were issued from the laboratories which were cooperating in the survey, and they were summarized in this journal (*Nutrition Reviews* **3,** *91 (1945)*). Since that time approximately two dozen papers have appeared, dealing with the contents of several "B vitamins," fat soluble vitamins, and minerals in canned foods, the reproducibility of assay values, and the effect of processing, storage, etc., on nutritive value. The first three of these will be discussed herein, whereas a later article (Part II) will deal with the factors affecting the nutritive value of foods.

The second year of the study was conducted in a manner similar to the first (*loc. cit.*). W. F. Hinman, M. M. Higgins, and E. G. Halliday (*J. Am. Dietet. Assn.* **23,** *226 (1947)*) reported that 351 carotene, 454 ascorbic acid, and 302 thiamine assays were carried out on 482 samples of thirty-two different vegetables and fruits that were collected from widely distributed areas and during different parts of the season. However, this number was much smaller than those assayed the first year (823) since greater attention was paid to the foods which were of greatest nutritional importance. Only minimal numbers of assays of thiamine, for example, were included in white corn, grapefruit, and pears. On the other hand, the number of samples of peas was increased, and eleven new foods were added to replace a number of relatively unimportant ones that were dropped from the survey.

Most of the ranges of carotene assay values exhibited a two- to fourfold spread, but despite this the variations were smaller than for the previous year for all foods except carrots. The average values for the two years were in good agreement. In addition to the carrots, spinach, and apricots that were first reported to be the best sources of carotene, four foods of the new list were found to contribute comparable amounts of carotene; these were sweet potatoes, turnip greens, peppers, and pimentos. Leading contributors of ascorbic acid were again the citrus fruits, although the content per gram in peppers and pimentos was far higher (110 to 120 mg. per 100 g. compared with 32 mg. per 100 g. of orange juice). The agreement between average ascorbic acid values for the two years was good, and although the ranges lay within a one and a half- to threefold spread, these ranges were much more uniform than those encountered during the first year. Uniformity of values was best among the thiamine determinations, where only six of the thirty-two products showed more than a twofold spread. Agree-

ment between the averages for the two years was within 15 per cent with most products. Peas and tomatoes were considerably richer in thiamine than most other foods tested.

The same foods, together with sardines, were examined for riboflavin, niacin, and pantothenic acid (M. Ives *et al.*, *J. Am. Dietet. Assn.* **21,** *357* (*1945*)) and the results were also found to be in excellent agreement with those of the initial survey. Variations among determinations were again high. The contents of niacin in most of the foods were considerably higher than of the other two vitamins, and pantothenic acid in turn was present at two to ten times the extent of riboflavin.

Values for pyridoxine, biotin, and folic acid were added by M. Ives, A. E. Pollard, C. A. Elvehjem, and F. M. Strong (*J. Nutrition* **31,** *347* (*1946*)). The extent of this testing program was somewhat restricted, embracing only nine fruits and vegetables, and salmon. Nine to eleven samples of each food were tested, each sample consisting of an aliquot from six consumer-size cans or one to three No. 10 cans. The solids and liquids were analyzed separately to determine the distribution of the vitamins in each portion, but the total contents were reported on the basis of the original contents of the cans, except for salmon, from which the brine was discarded. Pyridoxine was assayed by the yeast method of Atkin, Schultz, Williams, and Frey; biotin, by the microbiologic method of Shull and Peterson; and folic acid with Lactobacillus casei by the method of Teply and Elvehjem and with Streptococcus faecalis by the method of Luckey, Briggs, and Elvehjem. Crystalline pteroylglutamic acid was used as standard.

Pyridoxine contents were much higher than those of biotin or folic acid. Tomatoes, spinach, and yellow corn were the richest vegetable sources, with 60 to 70 micrograms per 100 g., but salmon contained 130 micrograms. Salmon (9.8 micrograms per 100 g.) was likewise the best source of biotin, being four times as rich in this vitamin as the vegetables, all of which contained 1.3 to 2.3 micrograms. Spinach, as expected, was the best source of folic acid according to both assay methods employed; an average value of 20.7 micrograms per 100 g. was obtained with L. casei, whereas salmon and the other vegetables ranged from 4.1 to 9.0. The fruits tested were relatively poor sources of all three vitamins. Although the folic acid assays may likely be in error due to failure of takadiastase and papain to release bound forms of the vitamin; the relative figures are of value in indicating richer sources.

Pyridoxine and folic acid were distributed to the extent of approximately two-thirds in the solids and one-third in the liquids, which approximated the percentages of the total weight contributed by each phase. Biotin was somewhat less soluble than the other vitamins, since the amounts contributed by the solids ranged from 68 per cent in tomatoes to 99 per cent in spinach.

Information concerning the proximate and mineral composition of canned foods was furnished by A. Kramer (*Food Research* **11,** *391* (*1946*); *J. Am. Dietet. Assn.* **21,** *354* (*1945*)). A total of 1384 samples was analyzed. The methods of the Association of Official Agricultural Chemists (1940) were used throughout, except for the author's modification of Stugart's method for iron. Fruits were notably low contributors of protein and fat, and with a few exceptions were also low in phosphorus. They were high in carbohydrate (12 to 25 per cent), and if their generally high water content is considered, most of them were fair sources of calcium (approximately 20 mg. per cent) and iron (blueberries contained 6 mg. per cent). As expected, the fish products were the best sources of protein, fat, calcium, and phosphorus. Vegetables gave generally intermediate values, with the exception that they were uniformly low in fat. The seed vegetables were high in phosphorus, and fairly high in protein and calcium. The protein content of many foods paralleled the total solids content.

The distribution of these nutrients between the solid and liquid portions of canned foods varied widely, so that few, if any, valuable generalizations could be made. The percentage of the total nutrients present in the liquid portion was seldom greater than the percentage of total weight contributed by the liquid, and insoluble fractions such as fat and fiber, were much lower in the liquid portion. It was felt that the large variations (fourteenfold in the iron values, that increased during storage, indicating that iron was removed from the can) might be due in part to the style of pack or the degree of maturity of the food; thus conversion of polysaccharides to simple sugars would be reflected in a higher content in the liquid portion.

Possible reasons for the wide variations have received special attention by N. B. Guerrant, M. G. Vavich, and O. B. Fardig (*Ind. Eng. Chem.* (*Anal. Ed.*) **17,** *710 (1945)*), who submitted pureed samples of four vegetables, salmon paste, and finely chopped ham in eight- and twelve-ounce tins to twenty-one collaborating laboratories. The various samples were analyzed for carotene, ascorbic acid, thiamine, riboflavin, pantothenic acid, and niacin, with four to nine laboratories reporting on each vitamin, according to the methods that they were commonly using. From the results obtained it was evident that at least a part of the variation could be ascribed to poor agreement among the assays or the assaying laboratories. Thus, for example, the carotene values on corn varied from 0.08 to 0.38 mg. per 100 g., and the riboflavin values on peas varied from 0.072 (microbiologic) to 0.400 (fluorometric) mg. per 100 g. Agreement between values for the salmon paste and chopped ham were generally poor, whereas those on pureed vegetables were usually better; thiamine and niacin values on these foods were in good agreement, and the microbiologic riboflavin assays agreed rather closely. Since variations in assay methods would not normally be found in a report from an individual laboratory, it would seem that lack of uniformity of product might have contributed to the large spread in values reported by the previous investigators. In addition, the present authors felt that human variability was an important factor, although no supporting evidence was offered. Experience over a period of years with collaborative assays of other vitamins has generally been toward greater uniformity of results than was encountered here. If product variability is to be regarded as a major cause of nonuniformity among assay values of canned foods, then only the most approximate averages can be employed, even when many samples are tested. The fact that the average values for several of the vitamins were in close agreement during two successive years probably indicates that the number of samples was large enough to justify the approximate values listed.

NUTRITIVE VALUE OF CANNED FOODS (PART II)

Examination of canned foods during storage reveals a gradual deterioration of vitamin values, despite the progress that has been made in lessening such losses. N. B. Guerrant, O. B. Fardig, M. G. Vavich, and H. A. Ellenberger (*Ind. Eng. Chem.* **40,** *2258 (1948)*) and B. B. Sheft, R. M. Griswold, E. Tarlowsky, and E. G. Halliday (*Ibid.* **41,** *144 (1949)*) have reported these changes in carotene, ascorbic acid, thiamine, riboflavin, and niacin in canned vegetables and fruits stored over a two year period at 50°, 65°, and 80°F. Losses were somewhat greater at 80°, especially of ascorbic acid and thiamine; retention of ascorbic acid after storage for two years ranged from 48 per cent in apricots to 79 per cent in green asparagus. Comparable retentions of carotene ranged from 63

per cent in peaches to 98 per cent in tomato juice; riboflavin, 27 per cent in green beans to 97 per cent in tomatoes; thiamine, 46 per cent in asparagus to 86 per cent in peaches; and niacin, 71 per cent in asparagus to 99 per cent in peaches. Losses were less at the lower storage temperatures, and the larger losses observed for riboflavin and ascorbic acid were often appreciably less after storage for only twelve months. Previously, N. B. Guerrant, M. G. Vavich, and R. A. Dutcher (*Ibid.* **37,** *1240 (1945)*) had found that retention of ascorbic acid and thiamine in tomato juice and vegetables was only 20 per cent after storage for one year at 110°F., whereas storage at 42°F. or 30°F. had little effect on these or three other vitamins. It seems clear from these reports that the best nutrition practices should include warehousing at low temperatures, together with regular consumption of each season's pack—a factor which may be of even greater importance to housewives than to commercial packers.

Several studies have been made of changes in the nutritive value of foods during processing. A general survey was carried out by N. B. Guerrant *et al.* (*J. Nutrition* **32,** *435 (1946)*) which included determinations of five vitamins in nine vegetable foods and cherries, before and after canning. In several of the foods, observations were made after each of the four or five steps in the canning process. In general, vitamin retention was good in most of the foods tested, with the exception of beans and peas, in which losses of over 50 per cent were common, not only in ascorbic acid and thiamine, but also in riboflavin and niacin. Much of the losses appeared to occur in the blanching operation, as was borne out in later studies of blanching losses by N. B. Guerrant *et al.* (*Ind. Eng. Chem.* **39,** *1000 (1947)*) and by J. R. Wagner, F. M. Strong, and C. A. Elvehjem (*Ibid.* **39,** *985, 990 (1947)*). When peas, beans, corn, asparagus, and spinach were blanched in water for from one and one-half to twelve minutes at temperatures ranging from 150° to 200°F., or in steam for one to six minutes, retention of ascorbic acid dropped to 40 per cent in lima beans, depending upon the temperature and duration of the blanch, although in asparagus up to 94 per cent was retained. Similar extremes in retention of thiamine were 36 per cent in lima beans and 99 per cent in asparagus; for riboflavin, 40 per cent in peas and 103 per cent in asparagus; niacin, 51 per cent in lima beans and 98 to 100 per cent in the same product after a short period of blanching. The effect of blanching for periods longer than six minutes and above 160°F. was pronounced for all vitamins studied except ascorbic acid, where in green beans, retention after blanching two minutes at 210°F. was 69 per cent compared with 44 per cent at 160°F. The authors felt that the lower temperature may have favored oxidation, presumably due to less rapid exhaustion of air from the cans. After nine minutes, retention of water soluble vitamins was uniformly low. By contrast, losses of carotene were negligible, and it appeared that leaching of the other vitamins by the blanching water, especially from the small-particle vegetables such as lima beans and peas, was a major cause of the losses. Guerrant and co-workers found steam blanching to be more successful, for losses of ascorbic acid in green peas after six minutes were only 26 per cent. However, the data of Wagner, Strong, and Elvehjem revealed little difference between water and steam blanching. Attempts to reduce losses of ascorbic acid in peas through repeated use of the same blanching water were also of no value; retentions after blanching for six minutes varied from 67 per cent in the first batch to 72 per cent in the sixth. It would appear that a fairly constant amount of this vitamin is readily extracted by water.

A. Kramer and M. H. Smith (*Ind. Eng. Chem.* **39,** *1007 (1947)*) extended the studies on blanching to the proximate and mineral composition of vegetables. The leaching that occurred with the various vitamins was also noted here, with the extraction of soluble

solids increasing with the blanching time. Losses of carbohydrate in spinach after blanching for seven minutes were approximately 30 per cent, whether this was carried out at 170°, 185°, or 200°F. Loss of carbohydrate was only 10 per cent in peas and lima beans and about 5 per cent in green beans. Losses of protein were slight, reaching a maximum of about 10 per cent in peas. Minerals, on the other hand, were extensively leached, especially from spinach, where a 54 per cent loss of total ash was incurred during blanching for seven minutes at 200°F. Losses of phosphorus were high in this vegetable, reaching 40 per cent under the most severe conditions, but calcium was taken up from the blanch water both by spinach and peas, so that increases of 54 and 79 per cent in the calcium content were obtained. The over-all loss in ash content (from 1.74 to 0.80 per cent after seven minutes) must therefore have been due chiefly to diffusion of potassium salts into the blanch water. Steam blanching produced only minor changes in all vegetables with the exception that approximately 20 per cent of the carbohydrate was removed.

The over-all program of the National Canners Association and the Can Manufacturers Institute, which has been carried through by the various cooperating laboratories, has resulted in a better understanding of the contributions made by canned foods to national nutrition, and it may be said that more is known about the nutritive values of canned foods than about any other type of processed food.

CASTLE'S EXTRINSIC FACTOR AND VITAMIN B_{12}

Pernicious anemia is characterized by a greatly decreased secretion of gastric juice containing no free hydrochloric acid even after the maximum stimulation provided by histamine. That the hematologic defect in the disease is etiologically related to achylia gastrica (absence of gastric juice) has been clearly established. The classic researches of W. B. Castle (*Am. J. Med. Sci.* **178,** *748 (1929)*) demonstrated that feeding 200 to 300 g. of finely divided beef muscle to patients with pernicious anemia was without effect on blood formation. Subsequent feeding of the same quantity of beef previously incubated with normal human gastric juice resulted in unmistakable evidence of hematopoiesis. Castle hypothesized, therefrom, that the normal stomach secretes some factor which is absent from or greatly reduced in the stomach of the patient with pernicious anemia. Feeding normal human gastric juice alone is ineffective in this disease (W. B. Castle and W. C. Townsend, *Ibid.* **178,** *764 (1929)*). These and other studies led to formulation of the hypothesis that pernicious anemia "is a 'conditioned' defect of nutrition. Thus, patients suffering from pernicious anemia are seemingly unable to derive from food some substance essential for normal function of bone marrow. The nutritional defect in such patients is apparently caused by the failure of a reaction which occurs in the normal individual between a substance in the food (extrinsic factor) and a substance in the normal gastric secretion (intrinsic factor)" (W. B. Castle and T. H. Ham, *J. Am. Med. Assn.* **107,** *1456 (1936)*). It was further hypothesized that liver contains a "liver extract principle" which is capable of restoring hematopoiesis in pernicious anemia. Accumulation of this principle in the liver is dependent upon the normal interaction of extrinsic and intrinsic factors. The exact relation between the extrinsic, intrinsic, and liver factors has not been clearly defined, but it has been widely held that the extrinsic and liver factors were distinct substances.

For a long while it has been known that the antipernicious anemia potency of liver

extract could be increased by incubation with gastric juice (F. Reimann and F. Fritsch, *Z. f. klin. Med.* **126,** *469 (1934)*; P. J. Fouts, O. M. Helmer, and L. G. Zerfas, *Ann. Int. Med.* **8,** *790 (1935)*). These observations have been interpreted as showing that liver contains both extrinsic factor and liver extract principle.

New light is shed on these relations by an investigation of vitamin B_{12} (L. Berk *et al.*, *New Engl. J. Med.* **239,** *911 (1948)*). These workers confirmed the earlier findings of others regarding the potentiation of liver extract by gastric juice. They fed 15 units of a highly purified liver extract to a patient with pernicious anemia. A slight reticulocytosis occurred. Subsequently, simultaneous administration of the same amount of liver extract plus normal human gastric juice resulted in a higher reticulocytosis than had followed the liver extract alone.

Four patients were then fed a diet free of (or low in) extrinsic factor. All were given 5 micrograms of vitamin B_{12} orally. Three received, in addition, 150 ml. of neutralized normal human gastric juice twelve hours after ingestion of the vitamin B_{12}. Over a ten day period there resulted slight, if any, hematologic response. For the following ten days the 5 micrograms of vitamin B_{12} and the normal gastric juice were given simultaneously. In the second period a greater hematopoietic effect was noted in 3 of the 4 patients. This effect was evident from both a higher second reticulocyte peak and more rapid erythropoiesis.

These observations indicate that the hematopoietic activity of vitamin B_{12} in pernicious anemia is potentiated by normal human gastric juice. Since this is the property whereby the extrinsic factor is characterized, it is apparent that vitamin B_{12} may be considered as identical with or similar to the food (extrinsic) factor of Castle. A further implication of these studies may be that liver does not contain both a "liver principle" and "extrinsic factor" as previously assumed, but, rather, that the observed potentiation of liver extract by gastric juice is but the increase in activity of the vitamin B_{12} (extrinsic factor) in the liver. Perhaps the "liver principle" and the "extrinsic factor" are identical or very nearly so. It is worth recalling at this point that E. L. Smith (*Nature* **161,** *638 (1948)*) concentrated from liver two related red pigments, both hematopoietically active in pernicious anemia but differing from each other in that one was acidic, the other neutral.

Berk *et al.* (*loc. cit.*) state that microbiologic assay of the vitamin B_{12} fecal loss by patients with pernicious anemia is large. Independent studies by F. H. Bethell, M. C. Meyers, and R. B. Neligh (*J. Lab. Clin. Med.* **33,** *1477 (1948)*) have revealed similarly large losses of vitamin B_{12} in the stool of patients with untreated pernicious anemia. It seems reasonable, therefore, to entertain the hypothesis that gastric juice potentiates the activity of orally administered vitamin B_{12} due to some favorable effect which it exerts on the absorption of the vitamin or of closely related substances in food. An equally attractive hypothesis would appear to be that normal gastric juice protects vitamin B_{12} (extrinsic factor) from destruction in the upper gastrointestinal tract and thereby affords a greater opportunity for its absorption.

Whatever may be the ultimate solution of this challenging problem, it is impossible to review critically Castle's numerous meticulously designed, tedious, and difficult clinical investigations throughout the past two decades and not remark on their design. Investigators in the basic sciences have often, with some justification, expressed concern for the lack of clear design and control in some clinical research. A review of Castle's studies demonstrates that some clinical investigations may be as well designed and controlled as other biologic researches.

FAT-GALACTOSE RELATIONS

The effect of vitamins on the utilization of food is thoroughly recognized, but other food components may also have mutual relations which affect their utilization. With regard to the latter, the experiments of the Wisconsin group (*Nutrition Reviews* **1,** *122, 358 (1943)*; **2,** *165 (1944)*) on the relation of butterfat and lactose may be recalled. A difference between butterfat and vegetable fat in the growth of young rats was observed, and it became clear that this difference was observable only when lactose was the carbohydrate component of the diet, and probably only when this was at a high level in the diet such as would occur in the young animal. The difference between the two types of fat was not demonstrable at lower levels of lactose in the diet (*Ibid.* **2,** *267 (1944)*), and it has been suggested that butterfat gives a better growth response than other fats only when the lactose is so high in the diet that it is toxic (*Ibid.* **4,** *140 (1946)*). The toxic effect and difficult utilization of galactose are recognized (*Ibid.* **4,** *204 (1946)*; **6,** *185 (1948)*), and the effect of fat in causing better utilization of galactose has been indicated by E. J. Schantz, C. A. Elvehjem, and E. B. Hart (*J. Biol. Chem.* **122,** *381 (1938)*).

C. P. Richter (*Science* **108,** *449 (1948)*), by use of his "single-food-choice technique" has demonstrated the reverse relation, namely that galactose appears to have a marked positive effect on the utilization of fat. In this technic animals are offered only a single food and water, and their survival time is taken as a measure of the nutritive value of the foodstuff. The method has been extended to include two test substances, and its usefulness has been illustrated by experiments in which glucose alone or glucose and thiamine were offered. The latter animals survived an average of seventy-six days as compared with thirty-seven days for the former. In the experiments now reported, galactose, a vegetable oil "oleo," and both were offered to rats. With no food offered, 15 rats survived from three to six days, an average of four and three-tenths days. A similar group survived only slightly longer, six and two-tenths days, when offered galactose; when offered oleo the average survival time was thirty days. However, when separate containers of galactose and oleo were offered, the survival ranged from forty-seven to ninety-two days with an average of sixty-nine days. Thus one of the components had a beneficial effect on the utilization of the other.

A comparison of the actual amounts of galactose and oleo consumed strongly indicates that this sugar improves the utilization of oleo. Thus the galactose contributed only 15 per cent of the calories consumed, and some rats consumed the galactose at only irregular intervals. Rats offered galactose and oleo consumed more oleo than those with access to oleo alone. In other experiments a mixture of oleo and galactose (9:1 or 19:1) gave the prolonged survival time, but when glucose was used instead of galactose, survival time was not greater than with oleo alone. Thus the effect of galactose appears to be specific.

In discussing their experiments, the author points out that the effects observed were with small amounts of galactose. Experiments are cited in which a high proportion of galactose in the diet has been toxic, and might explain the results of B. H. Ershoff and H. J. Deuel (*Am. J. Physiol.* **148,** *45 (1947)*) in which no effect of galactose on fat utilization was observed on a diet containing 70 per cent galactose. As to the possible mechanism of the galactose effect, the marked antiketogenic effect of galactose as compared with glucose and fructose was mentioned, as well as the pronounced nitrogen-sparing action that has been recorded for galactose. The author seems to favor a more specific but as yet unknown metabolic effect of galactose.

Provided that the experiments are con-

firmed with other fats, and that galactose is superior to other sugars, the author suggests that fortification of edible fats and oils with galactose be considered for the diet of man. It must be recognized, however, that Richter's experiments were conducted under very specialized conditions, and only if an effect of galactose on fat utilization is demonstrable on a mixed diet, would the above suggestion be considered seriously.

THIAMINE DEFICIENCY IN THE RHESUS MONKEY

J. F. Rinehart, L. D. Greenberg, and L. L. Ginzton (*Blood* **3,** *1453 (1948)*) using a diet similar to that of H. A. Waisman and K. B. McCall (*Arch. Biochem.* **4,** *265 (1944)*) studied the effects of thiamine deprivation in 7 monkeys (Macaca mulatta). This diet consists of sucrose, vitamin-test casein, salt mixture, corn oil, and sulfited liver extract. The last furnishes biotin and folic acid. In addition, vitamin tablets were given that contained niacin, riboflavin, pyridoxine, calcium pantothenate, choline, para-aminobenzoic acid, inositol, and ascorbic acid. Some animals also received mixed tocopherols.

The monkeys were carefully selected and were free of tuberculosis. They were first placed on the purified diet with complete supplementation for one or two weeks, including 1 or 0.5 mg. of thiamine chloride daily. Control tests were carried out during this time so that each monkey could serve as his own control. The occasional animal that failed to adapt itself to this diet or failed to gain in weight during the control period was rejected. With the exception of one, an older animal weighing 7000 g., the monkeys weighed between 1800 and 3600 g. at the initiation of the studies.

The clinical behavior of the animals was in most respects similar to that described by H. A. Waisman and K. B. McCall (*Nutrition Reviews* **3,** *21 (1945)*). In general, the monkeys ceased gaining weight after two weeks on the thiamine deficient diet. This was followed either by a plateau of the weight curve for several days or by loss of weight. The weight loss was usually associated with decreased food consumption and marked lowering of the blood thiamine, as measured by the technic of L. D. Greenberg and J. F. Rinehart (*Proc. Soc. Exp. Biol. Med.* **59,** *9 (1945)*). As the deficiency progressed, the animals continued to lose weight, became apathetic and inactive, and weakness was evident. Finally, they became ataxic and some developed ptosis and tremors. Retching was observed but vomiting did not take place. If thiamine was administered at this stage, a dramatic response was observed in twenty-four to forty-eight hours, striking improvement in locomotion, alertness, and appetite being evident. On the other hand, if the period of thiamine deprivation was not interrupted at this stage, it was but a matter of a few days until the monkey was unable to sit on its perch or even stand upon the floor of the cage without difficulty. At times there appeared to be a paralysis of the hind legs, the animals climbing or moving in the cage only by the use of the forelegs. Edema was observed in only one animal during the period of depletion. However, following administration of thiamine to acutely deficient animals, edema was noted in several instances.

The control animals, on the other hand, continued to gain weight, although not as rapidly as animals on a stock diet, and remained strong and healthy during the course of the experiment. It is stated that control animals have been maintained on the complete diet for a year or longer without any obvious alteration in their strength or vigor. One such animal was killed and autopsied after having been on the diet for a period of six months and no pathologic changes were in evidence during either gross or microscopic examination of the tissues.

Blood thiamine levels ranged from 5.5 to 10.0 micrograms per 100 ml. of whole blood during the period when the monkeys were on the complete diet, including the thiamine supplement. Upon withdrawal of the thiamine, the blood level usually dropped to values of 4 micrograms or less and, except for some minor fluctuations, remained low.

An attempt was made to estimate the minimum thiamine requirement of the monkey by observing the time required to redeplete an acutely deficient animal following administration of a small dose of the vitamin. Assuming that the full dose of the vitamin given by mouth was retained, this total dose was divided by the product of the elapsed time in days and the weight in kilograms. This value is regarded as the "minimum daily requirement" per kilogram of body weight. Such calculations in 4 monkeys, in one animal through four depletion periods, gave values which varied considerably. The average was 15.5 micrograms per kilogram per day, which is in close agreement with the figure reached by Waisman and McCall (*loc. cit.*).

Certain tissues were removed at the time of autopsy and subjected to analysis for thiamine and riboflavin. Compared with a control animal killed at the termination of the study, the data showed a marked lowering of the thiamine content of every tissue examined in the deficient animals; namely, skeletal muscle, brain (cortex), heart (ventricle), kidney, and liver. These values ranged from 10 to 30 per cent, approximately, of the control values. The riboflavin concentrations of the heart, kidney, and liver in the deficient animals were found to be slightly higher than those observed in the control animal. However, a greater concentration of riboflavin was observed in the skeletal muscle of the control animal than in the deficient ones.

Depletion for thirty days in the experimental animals was associated with a slight but definite reduction in the red blood cell count and hemoglobin. The reticulocyte count decreased, falling from 1 to 2.7 per cent to zero. When small subcurative doses of thiamine (200 to 500 micrograms orally or parenterally) were given, increases in reticulocytes up to 4.5 per cent occurred. At the same time, however, there was a fall in the red blood count and hemoglobin rather than a return to normal.

The authors conclude that thiamine deficiency in the rhesus monkey will cause anemia and that this is due to suppression of reticulocyte formation. Since this conclusion is contrary to observations in other species, such as the pig (M. M. Wintrobe *et al.*, *Bull. Johns Hopkins Hosp.* **71,** *141 (1942)*), it is unfortunate that Rinehart *et al.* fail to present more convincing evidence. Thus, as already indicated, administration of thiamine did not seem to lead to disappearance of the anemia. Again, their thesis that the anemia is attributable to suppression of reticulocyte formation is not supported by bone marrow studies.

Severe retrogressive changes in the nuclear structures of the central nervous system in thiamine deficient monkeys have been described in preliminary reports by J. F. Rinehart, L. D. Greenberg, and M. Friedman (*Am. J. Path.* **23,** *879 (1947)*; *Tr. Am. Neurol. Assn., p. 174, William Byrd Press Inc., Richmond, Virginia (1946)*). The lesions in the cranial nerve nuclei and other nuclei in the midbrain, pons, and medulla resembled the lesions of Wernicke's disease although the vascular changes commonly described in that condition were seen only occasionally, in the later stages. The distribution of the lesions also differed from that seen in Wernicke's disease and altogether the findings were remarkable in the way they differed from observations in the nervous systems of thiamine deficient animals of other species (*Nutrition Reviews, loc. cit.*).

A COMPARISON OF TECHNICS OF DIETARY STUDY

Several technics have been widely employed in efforts to determine food consumption and nutrient intake of populations. In view of the frequent application of these methods, it is surprising to find that they have seldom received critical study. It is probable that no other set of methods has so frequently served as a basis of "conclusions" prior to exact definition of reliability of the findings as have dietary studies.

A straightforward approach to the evaluation of different technics of individual dietary study has been made by E. R. Bransby, C. G. Daubney, and J. King (*Brit. J. Nutrition* **2,** *89 (1948)*). This approach consisted of applying four methods of individual dietary survey to a group of 49 children in a children's home. The methods applied were weighing, questioning, homely measures, and chemical analysis. The diets of 49 boys, 10 to 15 years of age, in the National Children's Home were studied by all of these methods for a single three day interval. The food served to each subject was measured by household measures (slices, cupfuls, teaspoonfuls, etc.) and recorded to provide the "homely measures." The same servings were then weighed (weighing technic) and duplicated, the duplicate meal being used for analysis. Each evening the children recalled the food consumed during the day (questioning). Nutrient calculations were based on modern food tables widely used in England. Analyses of the duplicated meals were made for moisture, ash, iron, calcium, nitrogen (Kjeldahl), fat, "available carbohydrate," sugar, and starch. The analytical methods employed were reasonably standard procedures which are adequately described by the authors.

The results showed revealing and often unexpected differences in appraisals of consumption by the several technics. The well tabulated comparisons may be illustrated by a few examples and generalizations:

Compared with the values obtained by chemical analysis, the other three methods overestimated the average consumption of all nutrients except iron, for which there was an underestimate, and for protein, for which weighing and questioning underestimated and homely measures showed no difference. Differences between average consumption of foods estimated by weighing and homely measures were 26 per cent for meat, bacon, and fish; 31 per cent for meat or fish pies; 15 per cent for puddings; 29 per cent for fruit; and 31 per cent for eggs. In most instances the use of homely measures resulted in an overestimate of the amount of food eaten. On the other hand, the questioning procedure generally resulted in an estimate of intake considerably less than the weighed intake.

The variation between average daily intakes of nutrients as estimated by the four technics may be illustrated by the calculated mean ($\pm$ S.D.) intake derived by weighing, homely measures, questioning, and chemical analysis, respectively: calories, 2660 $\pm$ 507, 2771 $\pm$ 423, 2607 $\pm$ 487, 2381 $\pm$ 218; fat, 96 $\pm$ 22, 107 $\pm$ 21, 95 $\pm$ 19, 81 $\pm$ 14; iron, 15 $\pm$ 3.3, 16 $\pm$ 3.9, 15 $\pm$ 3.1, and 19 $\pm$ 3.1. Except for fat and iron, the correlation coefficients were reasonably high (0.71 to 0.97) between nutrient values obtained by the several methods, indicating that ranking of the values by the four methods is approximately the same.

Disturbingly great differences obtained between the calculated values for nutrients in individual diets as compared with the values found on analysis. Thus, for forty-two of the forty-nine dietaries the results for calories as estimated by homely measures exceeded the chemically determined values by more than 10 per cent, and in sixteen of the forty-nine dietaries by more than 20 per cent. Even greater percentage differences were observed between the estimates of intakes of fat, iron, and calcium.

The conclusion is justifiable that while

average values arrived at by the several technics tend to be of the same order of magnitude, and with certain exceptions the relative ranking of values is somewhat similar, there may exist little similarity between the true nutrient consumption of an individual and that estimated for the individual by any of the technics short of actual chemical analysis. This finding again brings forth the futility of attempts to assess from dietary data alone the incidence of deficiencies within a population.

In an additional field study of the diets of the schoolchildren as revealed by weighing and questioning, these authors found similar variations. This second investigation included an examination of the effect of memory aids (lists) with the questioning technic in improving the agreement between the estimated and weighed intakes. Memory aids did not improve the reliability of these estimates.

The results of this study, together with the other few published isolated critical investigations of validity of dietary methods, show clearly that technics of study of dietary intakes which are suitable to large-scale application can do no more than depict the broad outlines of food or nutrient intake of the population. The question may be asked whether elaborate efforts at refinement of such technics are justifiable. It is certain that the methods cannot be employed to provide valid estimates of the incidence of deficiency states within a population. Further, the technics short of actual analyses of intake cannot serve alone to categorize sharply segments of the population according to actual level of nutrient consumption.

With the data now available indicating the value and limitations of methods of dietary study, it seems reasonable to accept these appraisals and to recognize that efforts to refine the methods have not resulted in the development of precise methods applicable to large groups. As efforts are made at further refinement of such methods it is hoped that workers will follow the suggestion of Bransby and co-workers that " . . . before any new survey method is used, it should undergo field trials, the results of which should be published. Data should be included to show, for example, whether a method is suitable simply to ascertain the broad levels of consumption, or whether it can be used for more precise work, and to what population groups it can be applied." In the meantime, it seems reasonable to seek the simplest and cheapest method of dietary study which will provide the broad picture of food consumption and to hope that biochemical or other approaches can be developed for appraisal of nutritional status of individuals.

GASTRIC UREASE

Physiologists have been interested for many decades in gastric secretion and the ability of parietal cells of the mucosa to produce a secretion of hydrochloric acid which is about tenth-normal. The properties of mucosal cells which protect against digestion in secretions of such high acidity are almost without parallel in biology. There is a somewhat similar situation in certain of the arthropods in which salivary glands produce secretions of sulfuric acid of relatively great acidity. In gastric physiology saliva entering with food through the esophagus, gastric mucin, and intestinal juices regurgitating through the pylorus have been demonstrated to contribute to the neutralization of the highly acid gastric juice.

The mucosa of the stomach of certain mammals was shown by J. M. Luck (*Biochem. J.* **18,** *825 (1924)*) to effect enzymatic hydrolysis of urea, forming relatively large quantities of ammonia. Urease was found to be contained primarily in the superficial cells of the mucosa of the fundus and pylorus

(K. Linderstrøm-Lang and A. S. Ohlsen, *Enzymologia* **1,** *92 (1936)*). In the reports of O. FitzGerald (*Nature* **158,** *305 (1946)*) and FitzGerald and P. Murphy (*Ibid.* **162,** *896 (1948)*) evidence is provided that urease may have an important role in the gastric physiology of man and that the ammonia formed may contribute significantly to neutralization of the highly acid gastric juice.

Variations were observed in the urease activity of the stomach mucosa of different species. The activity of this enzyme was greater in man, dogs, and cats than in rabbits, pigs, and rats. Greatest quantities were present in the fundus, less in the pylorus, and relatively small amounts in the small intestine. It was demonstrated that the whole mucosa of a single cat stomach incubated at room temperature elaborated sufficient ammonia to neutralize 47 ml. of tenth-normal hydrochloric acid, or much more acid than is secreted in the cat stomach under any circumstance.

Although there is great variation of concentrations of urease in the stomach of different species, little variation in concentration is found among individuals of the same species on similar diets. However, with changes in the diet marked differences occurred in urease activity within a given species. Cats fed a meat diet were found to have concentrations of gastric urease six times as great as those of cats on a milk diet. Similarly when soya bean flour in which naturally occurring enzymes had been inactivated was fed to rats there was a doubling of quantities of gastric urease found with control diets. The urease content of the intestinal mucosa remained unchanged with these dietary manipulations. Concentrated pregnancy urine injected into rats caused an increase in activity of the gastric enzyme. This effect was considered to be due to the presence in the injected material of relatively large concentrations of urogastrone.

The effects of feeding 15 g. of urea on the free acidity and the ammonia nitrogen of the stomach contents after injection of histamine were investigated in 14 normal human subjects. The mean content of hydrochloric acid following histamine was 60 milliequivalents in the absence of urea but only 27 milliequivalents after oral administration of urea. Concurrently there occurred an inverse change in the ammonia nitrogen of the stomach contents, with a mean value of 10 milliequivalents after the histamine injection when urea was not fed and 37 milliequivalents with urea feeding. The total acidity of the gastric contents was not determined but the volume of secretion produced following injection of histamine was unchanged when urea was present in the stomach. It is predicated that the ammonia released was an important factor in neutralization of the acid elaborated after histamine stimulation of gastric secretion. Similar results were observed when a test meal of gruel was provided.

If under normal conditions of digestion urease has an integral role in providing ammonia for neutralization of gastric acidity, the substrate, urea, must be made available by physiologic means. There is no information which would indicate urea synthesis within the gastric mucosa. It is probable, then, that the substrate is derived from urea circulating in the blood stream. It has been reported that the concentration of urea in venous blood of the stomach is less than that of arterial blood in states of uremia (R. Vladesco, D. Simci, and M. Popesco, *Compt. rend.* **192,** *308 (1931)*). It is not known whether a similar arteriovenous difference occurs in normal function. FitzGerald and Murphy calculate that a urea concentration greater by 5.5 mg. per cent in arterial blood would provide adequate urea for neutralization of the hydrochloric acid secreted during digestion of a test meal. Concentrations of blood urea have been observed to increase during digestion of foods containing protein. A postprandial increase in blood urea would make additional urea available for formation of ammonia in the gastric mucosa.

L. Martin (*J. Am. Med. Assn.* **100,** *1475 (1933)*) investigated the nonprotein nitrogen constituents of gastric secretions in various disease states and found no apparent change in concentrations of urea or of ammonia nitrogen in individuals with gastric ulcers. However, in pernicious anemia and in benign achlorhydria there was an increase in concentration of ammonia.

FitzGerald and Murphy administered urea in the treatment of peptic ulcer. From the data of these authors this approach to therapy may be considered reasonable and directed at alleviation of the hyperchlorhydria which accompanies the disease. Early results of this form of therapy have been promising. It will be of interest to await the more significant data available after prolonged observation of larger groups of patients.

SYNTHESIS OF FATTY ACIDS IN ADIPOSE TISSUE

The view that carbohydrates may serve as precursors for formation of body fats has resulted from a variety of investigations. The general assumption that the liver was the sole site of fatty acid synthesis has been questioned by DeW. Stetten and G. F. Grail (*J. Biol. Chem.* **148,** *509 (1943)*) who calculated that the quantity of fatty acids synthesized by the mouse per day was about four times the quantity present in the liver at any one time. Furthermore, they found that the rate of removal of the fatty acids from the liver was too slow to allow the assumption that the liver was the major site of fatty acid synthesis. An extrahepatic source was further indicated by the studies of J. Tepperman, J. R. Brobeck, and C. N. H. Long (*Yale J. Biol. Med.* **15,** *855 (1943)*) who demonstrated that fat synthesis occurred in the functional absence of the liver.

E. Tuerkischer and E. Wertheimer (*J. Physiol.* **104,** *361 (1946)*) suggested that adipose tissue might play an appreciable role in the synthesis of fat. They observed that when rats were fasted or given inadequate diets until depot fat was exhausted and then given high carbohydrate diets, glycogen was initially deposited in the fat cells. After a definite interval had elapsed, the glycogen gradually disappeared with a simultaneous increase in fat. The respiratory quotient of isolated glycogen-containing adipose tissue incubated in serum was found to be 1.1 to 1.3, whereas the customary glycogen-free adipose tissue had a respiratory quotient of 0.70 when incubated under identical conditions (A. Mirski, *Biochem. J.* **36,** *232 (1942)*).

B. Shapiro and E. Wertheimer (*J. Biol. Chem.* **173,** *725 (1948)*) have presented direct evidence to substantiate the premise that adipose tissue was capable of synthesizing fatty acids. Samples of adipose tissues from rats were incubated at 37°C. for four hours *in vitro* in rat serum which had been enriched with deuterium oxide (heavy water) at a level of 10 atom per cent. The rats used had been maintained (1) on a stock diet ad libitum, (2) on a restricted intake until a 20 per cent loss in body weight had resulted, followed by the ad libitum feeding of a high carbohydrate diet for forty-eight hours before the synthesis test, or (3) on a restricted intake until a 30 per cent body weight loss occurred, followed by refeeding for sixteen hours. The last two procedures were used to cause high rates of fat synthesis. After incubation of the samples, that had been selected from the mesenteries, the interscapular region, and the groin, the fatty acids were released from the fats by saponification and acidification. The fatty acids were extracted with ether and specially prepared for determinations of their deuterium content.

The results indicated that deuterium had been introduced into the fatty acid molecules by this procedure. In general, the amount of deuterium incorporated into the fatty

acids was greater in the case of samples from rats which had been starved and then refed a high carbohydrate diet to increase the rate of fat synthesis. Parallel studies with the incubation of rat liver slices in serum containing deuterium oxide indicated that the fatty acids in liver incorporated deuterium at a much greater rate than the fatty acids being synthesized in adipose tissues. There was no comment as to whether any tests were made to determine whether an absolute increase in the total concentration of fatty acids occurred during the *in vitro* incubation period.

These *in vitro* studies strongly suggest that adipose tissues are capable of synthesizing fatty acids but permit no exact evaluation of the status of adipose tissues in the production of fats in situ in the body. The low rate of synthesis indicated in these studies for the adipose tissues may be compensated for by their presence in large quantities in the animal body.

NUTRITION AND ARTERIOSCLEROSIS

Since approximately 1910, heart disease in all its forms has been the most frequent cause of death in the United States. In 1945, deaths from diseases of the heart numbered 424,328, which was considerably more than twice the mortality from the second most frequent cause of death, cancer. These data, taken from the paper by I. M. Moriyama and M. Gover (*Pub. Health Reports* **63,** *537 (1948)*), indicate, as these authors state, that heart disease offers a major challenge to society today. Moreover, in those individuals in whom heart disease is a primary cause of death, association with arteriosclerosis is by far the most important contributory cause (M. Gover, *Ibid.* **64,** *104 (1949)*).

It is incumbent upon those interested in nutrition as well as other medical scientists to endeavor to elucidate the factors leading to heart disease and arteriosclerosis and to provide means for their treatment. The influence of diet upon hypertension was recently reviewed (*Nutrition Reviews* **6,** *293 (1948)*). It was pointed out that restricting the diet produces dramatic changes in cardiovascular function and that reverse changes occur following refeeding of starving individuals, with the appearance of epidemic hypertensive disease. The relation of diet to arteriosclerosis has likewise been reviewed (*Ibid.* **6,** *99 (1948)*) in which it was pointed out that in the absence of experimental data clinicians have been given little help with the dietary management of patients with vascular sclerosis.

The term "arteriosclerosis" is an all inclusive one covering experimental work in animals, the occurrence of subintimal fat deposits in man in all the various locations in the body and in all ages, and, finally, the appearance of arteriosclerosis with calcium deposits and other pathologic changes in the larger and smaller vessels in man. Nothing is more needed with respect to this disease condition than a clearcut statement of the problem. Such has been attempted with admirable success by R. Gubner and H. E. Ungerleider (*Am. J. Med.* **6,** *60 (1949)*).

The basic pathologic lesion of arteriosclerosis is, as the authors point out, infiltration of the arterial vessel wall (intima and subintima) with lipoids. Following this, large or small amounts of calcium may be laid down, degeneration of some of the arterial layers may occur, and, more importantly, the continuity of the intimal wall may be broken allowing intravascular blood clots to form. These thromboses frequently are of size enough to occlude small blood vessels, leading to a lack of oxygen in the tissues beyond the occlusion. In addition, clots may break off and travel with the blood stream to small vessels which they occlude. The reasons for

the lipoid infiltration of the arterial intima must be sought.

The arterial vessel wall is made up of three main layers known as the intima, subintima, and media, and these are covered by a fourth and outer coating known as the adventitia. As the authors point out, the artery has its nutrition supplied from two sources; the intima, subintima, and part of the media are nourished from blood flowing within the vessel itself while the adventitia and the outer portion of the muscular media are furnished nutrients by small vessels penetrating the vessel wall from outside, the so-called vasa vasorum. The source of the lipoids deposited in the inner layers of the vessel is most likely the blood flowing in the vessel itself. The factors concerned are the intravascular filtration pressure, the permeability of the intima, and the ability of the subintimal tissues to remove material brought to it.

Regarding the intravascular filtration pressure, the arterial pressure itself is the dominant factor determining penetration of the endothelium. Thus penetration of such substances as cholesterol may be increased in the presence of a high arterial pressure and hasten the development of arteriosclerosis. This is, of course, a well known clinical fact. Little is known of the second important factor, that of the permeability of the endothelial membrane, although many factors are known to influence it. Permeability is increased by lack of oxygen and certain others factors and decreased, as the authors note, by thyroid hormone, calcium, ascorbic acid, vitamin P, thiocyanate, and caproic acid. Little is known of the importance of these factors in the development of human arteriosclerosis. How important ascorbic acid and vitamin P and its analogues will be in this regard remains for investigation. The conflicting results of its use in a number of conditions related to vascular damage have been discussed (*Nutrition Reviews* **6,** *301 (1948)*).

Quantitative and qualitative changes in the serum lipids must be given an important place in the study of the genesis of arteriosclerosis in addition to investigation of the intravascular filtration pressure and permeability of the arterial intima. Of the serum lipids the most important seems to be cholesterol. The three important reasons for considering cholesterol above the other lipids are presented by D. Seegal (*see Am. J. Med.* **6,** *106 (1949)*), who notes first that cholesterol occurs in considerable amounts in arteriosclerotic plaques and that it has been possible to produce arteriosclerosis by feeding excess cholesterol to certain animals, and, finally, that arteriosclerosis develops at an early age and with increased severity in such diseases as diabetes mellitus and chronic glomerulonephritis, which are often associated with hypercholesterolemia. Gubner and Ungerleider suggest that the simplest reason for the presence of cholesterol in arterial walls is the result of the movement of cholesterol from the plasma together with other plasma constituents through the vessel walls and it seems possible that such penetration occurs together with protein. Because of its close association with hypercholesterolemia and other aspects of cholesterol metabolism, the authors suggest that regulation of the blood cholesterol might offer the most promising approach to the prevention of arteriosclerosis. Survey of the known facts with regard to cholesterol metabolism and the regulation of blood cholesterol are of considerable importance. The source of body cholesterol is apparently twofold, synthesis in the liver and absorption from the gastrointestinal tract. As indicated by W. H. Batchelor (*see Ibid.* **6,** *106 (1949)*), cholesterol is so readily synthesized that controlling the food intake of cholesterol may have very little effect upon cholesterol metabolism in the body. Another aspect of cholesterol metabolism to be considered is its relation to liver disease.

As is well known, choline deficiency in animals leads to production of fatty livers and lipotropic agents increase the rate of

removal of cholesterol from the liver into the bile and thence the gastrointestinal tract (*Nutrition Reviews* **4,** *345* (*1946*)). However, attempts to lower blood cholesterol by administration of lipotropic agents have met with only limited success as pointed out by Gubner and Ungerleider. This is in part due probably to the prompt reabsorption of cholesterol from the gastrointestinal tract. This leads to the next possible means of controlling blood cholesterol, that of influencing its excretion into the gastrointestinal tract or slowing its absorption. Presumably the major portion of cholesterol excretion into the intestinal tract is a direct one, probably into the large bowel, with biliary excretion a poor second in terms of quantity. The dietary restriction of cholesterol has been almost entirely ineffective in lowering the blood cholesterol level concentration. Significant decreases in blood cholesterol have been observed, however, with such strict low fat diets as the Kempner rice diet (*Ibid.* **6,** *293* (*1948*)). This seems reasonable because fat not only is necessary for absorption of cholesterol but also its metabolism in the body may yield acetate available for the further synthesis of cholesterol by the liver. However, as Gubner and Ungerleider point out, dietary restriction of fat must be extreme before an effect on blood cholesterol may be anticipated. Other measures such as exclusion of bile from the intestinal tract or interfering with pancreatic enzymes necessary for absorption of fat and cholesterol are at present highly impractical but several studies indicate that more work may lead to fruitful results. As previously noted, the ingestion of eggplant is claimed to reduce, at least temporarily, the blood cholesterol level (*Ibid.* **4,** *127* (*1946*)).

Cholesterol is degraded by the bacteria in the large intestine and some of the products of such degradation are not absorbed, or at least cannot be reconverted to cholesterol. Hastening such degradation by biologic or chemical means seemed to the authors to offer possibilities for reducing cholesterol absorption.

Whether lipids can be removed from the arterial wall once they have reached this area or not is unknown although it is clear that the small atheromatous plaques normally appearing in infants disappear at an early age. This is likewise seen in animals that have been fed a high cholesterol diet. When the dietary cholesterol is discontinued, the cholesterol deposits in the intima gradually disappear. It is doubtful whether such a reverse flow of cholesterol can be made to occur in adults in the absence of a marked reduction of the blood cholesterol level. Further, once severe pathologic changes such as calcification and loss of continuity of the vessel wall occur, little hope can be held for reversing the process by dietary or any other means.

The statement of the problem of arteriosclerosis in man by Gubner and Ungerleider opens up a great many avenues of investigation into the etiology, pathogenesis, prevention, and treatment of arteriosclerosis in man. It is clear that to a large measure the problem is one of a nutritional and metabolic nature.

VITAMIN K TOLERANCE TEST

Synthesis of prothrombin is dependent on a functioning liver and an adequate supply of vitamin K (*Nutrition Reviews* **2,** *101* (*1944*)). In individuals showing a deficiency of prothrombin (prolonged "prothrombin time"), administration of vitamin K should cause the prothrombin time to return to normal if liver function is not impaired. This possibility has been recognized and utilized by many investigators as a measure of liver function and as an aid in the diagnosis of liver disease. However, like most

tests of liver function it seemed that liver impairment had to be fairly well advanced before the test was particularly helpful.

P. N. Unger, S. Shapiro, and S. Schwalb (*J. Clin. Invest.* **27,** *39* (*1948*)) have devised and tested (Unger, M. Weiner, and Shapiro, *Am. J. Clin. Path.* **18,** *835* (*1948*)) what they believe to be an improved "standardized" method of using vitamin K as an indicator of liver impairment. Their test differs in two respects from most of those previously described. Very large doses of vitamin K are used (76 mg. of Synkayvite "Roche" given intravenously for four successive days) and the prothrombin time is followed daily. Their method of prothrombin estimation employs diluted plasma which is more sensitive than whole plasma procedures. A positive test is one in which a prolonged prothrombin time does not return to normal, or becomes prolonged rather than shortened after vitamin K is given.

In 123 patients having a variety of diseases this test was correlated with other standard liver function tests and with the clinical diagnosis. In 30 patients the various tests were compared with data from biopsy, autopsy, or operation. Of the 123 patients, 49 showed negative tests (prothrombin time 45 seconds or less) and in only 2 was a disease present which is usually associated with impaired liver function. In this group five of forty bromsulfalein, and eighteen of forty cephalin flocculation tests were positive, nearly all of these being considered as false positives.

Ten patients showed doubtful tests (prothrombin time 45 to 47 seconds). Of these, 8 had diseases expected to show hepatic impairment. Two of 7 showed positive bromsulfalein and 4 of 9 showed positive cephalin flocculation tests. The remaining 64 all showed positive vitamin K tolerance tests (prothrombin time 47 seconds or longer). Of these, 48 had clinical liver disease, and 3 had no evidence of liver disease. Thirteen patients showed severe anemia or were aged over 75 years, both of which seemed to give positive tests. As in the other group, the bromsulfalein and cephalin flocculation tests did not correlate with the presence or absence of clinical liver disease as well as did the vitamin K test. In general, the more prolonged the prothrombin time after vitamin K, the more uniformly the tolerance test and the clinical diagnosis agreed.

These results would seem to indicate that this test may be a useful laboratory procedure, and may be somewhat more sensitive than existing standard tests. However, the data are open to an objection common to all studies of this type since the criteria for the validity of the test rest primarily on its agreement with the clinical diagnosis, which is not always correct, especially when judging the degree of liver impairment. It is of especial interest, therefore, to examine the results of their test in those cases where anatomic or histologic confirmation of the clinical diagnosis was obtained. In general, these results confirm the previous impressions although both false positives and false negatives are apparent. In addition, it seems fairly clear that although this test may be more sensitive than existing ones, it is still generally negative until liver impairment is moderately advanced.

It would be interesting to determine whether the use of large doses of vitamin K does actually impose a "load" on the prothrombin synthesizing mechanism as these authors assume. It is generally believed that vitamin K acts like most other vitamins in enabling certain biochemical reactions to occur. An excess of vitamin K will not lower an already normal prothrombin time (A. J. Quick, *J. Biol. Chem.* **133,** *lxxviii* (*1940*)). Excess vitamin K has been reported to cause a paradoxical lengthening of prothrombin time in rabbits (P. Meunier, H. Hinglais, D. Bovet, and A. Dreyfuss, *Compt. rend.* **210,** *454* (*1940*)) that might be related to the mechanism operating in some of the patients observed by Unger *et al.* (*loc. cit.*) who showed increases in prothrombin time following administration of vitamin K.

NOTES

Letter to the Editor

Dear Sir:

May I comment on your review entitled "Feeding Versus Starvation in Infantile Diarrhea" (*Nutrition Reviews* **7,** *3* (*1949*)). The reviewer gives a comprehensive and altogether fair summary of the work of Chung and of Chung and Viščorová which supplied evidence in favor of early feeding in that condition. There follows a critical analysis of the data, some of which leads the reviewer to conclude that the original interpretations are "fraught with uncertainty." In brief the criticisms, some of which are made by implication only are the following:

(1) metabolism experiments carried out in the summer are "notoriously variable" because of the uncertain factor of insensible water loss;

(2) metabolism periods of twenty-four hours are too short to be significant, particularly during a period of rapid clinical change;

(3) the lag of urinary excretion in a period of rapid intestinal evacuation tends to invalidate the data;

(4) the relation between retention and load (intake) had already been established by "numerous" previous metabolic studies of diarrhea in infancy;

(5) the cumulative balance method of calculating retentions should have been employed;

(6) the balance studies were carried out after the period of initial electrolyte repair, being atypical of electrolyte repair as a whole;

(7) the variations in fat absorption were within normal limits;

(8) negative nitrogen balance in acute diarrhea is of little importance anyhow because of the ease with which it is later repaired.

Let us consider them one by one.

(1) The assumption that the metabolic observations of Chung were made during the summer months is a gratuitous one on the part of the reviewer. One of the 6 cases was studied in June, and that during a cool spell. The other 5 were studied between November and March. The reviewer was perhaps confused with the clinical studies of Chung and Viščorová in Bratislava that were carried out during the summer.

(2) Metabolism periods of twenty-four hours are unquestionably short. This was done advisedly in order to make a comparison of therapeutic regimens before any substantial change in the severity of the disease occurred. The reviewer is incorrect in assuming a rapidly changing status on the patient's part at the time of the metabolic studies. It is explicitly stated in Chung's report that experiments in which there was evidence of a change in the patient's disease status before the completion of a second metabolic period were excluded from the study.

(3) The lag in urinary excretion in a short metabolism period is a valid potential objection. With such short periods urinary figures will to a limited extent represent values characteristic of the preceding period. It was for this reason that the feeding procedure was reversed in different cases, high feedings being given first in some instances and last in others. Since the relation between intake and absorption was evident regardless of which feeding was given first, it seems fair to conclude that this potential objection did not introduce any serious error.

(4) Your reviewer refers to numerous balance studies of diarrhea in infancy which had shown what is described as a metabolic tenet that "in the presence of normal kidneys and tissue deficit excretion and retention become a matter of load." The implication is that Chung's study lacked

originality. The reviewer appears to have missed the point that Chung's studies were designed to elucidate not excretion or retention but absorption. I am not aware of other studies of infantile diarrhea in which the relation of intake to absorption has been evaluated.

(5) (6) Pointing to the importance of retention as measured by the cumulative balance method your reviewer contrasts the unfavorable balances of sodium calculated from Chung's data with the more favorable ones obtained by Darrow who employed conventional pediatric therapy, recognizing, however, that Chung's observations were confined to the height of the diarrhea while those of Darrow extended into convalescence. Interesting data of Gamble and of Darrow are quoted that reveal favorable initial retentions of sodium followed by a recession or reversal of this trend as further cumulative retentions were studied. For this reason the data of Chung are deemed applicable to only one transient phase of the process of repair. May I point out a possible difference between the experiments quoted and those of Chung which permit a different interpretation of the stages of electrolyte repair. Initial parenteral electrolyte was administered for the briefest possible time—a matter of a few hours only—in Chung's study; in one instance it was not given at all, only dextrose and water being given parenterally. In Darrow's experiments on the other hand it was continued for days. One may perhaps be permitted the thought that the negative sodium balances encountered by Darrow as oral feeding replaced parenteral feeding can be attributed to overtreatment with sodium, a situation less likely to have occurred in the experiments of Chung where the intestinal barrier was by-passed for a far shorter time. The stages of repair referred to may thus be artificial, depending upon the intensity of parenteral therapy rather than intrinsic features of the recovery process.

(7) Your reviewer's comment that Chung's data on fat absorption suggest "no apparent defect in the fat absorption mechanism during acute diarrhea" is difficult to comprehend in view of the fact that Chung reported absorptions ranging from 27 to 72 per cent of the intake and the normal is known to be about 85 to 95 per cent.

(8) The question of the importance of nitrogen retention in acute disease is, of course, a contentious one to which there is no clear answer at the present time. Blithely dismissed in some quarters, in others it is a matter of real concern. To those with the latter viewpoint the improvement in nitrogen retention achieved by Chung will not seem wholly without value.

L. Emmett Holt, Jr.
Bellevue Hospital
New York, New York

The Gordon Research Conferences

The Gordon Research Conferences, sponsored by the American Association for the Advancement of Science, formerly known as the Gibson Island Research Conferences, for 1949, will be held again this summer at the Colby Junior College, New London, New Hampshire. From August 8 to 12 the Conferences will deal with the subject of "Vitamins and Metabolism" and Dr. E. E. Snell will serve as chairman. From August 15 to 19 the subject under discussion will be "Food and Nutrition." Dr. Daniel Melnick will serve as chairman.

Requests for attendance at the Conferences, or for any additional information should be addressed to W. George Parks, Director, Department of Chemistry, Rhode Island State College, Kingston, Rhode Island. From June 15 to September 1, 1949, mail should be addressed to Colby Junior College, New London, New Hampshire.

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